（**FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !**）

Human Malaria Pf/Pan Antigen Lateral Flow Assay Kit

Catalog No: E-HD-C107

20T/40T

Version Number:	V1.0
Replace version:	V1.0
Revision Date:	2022.10.31

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.vetassay-elab.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

The Malaria Pf/Pan Antigen Test Kit is a lateral flow chromatographic immunoassay. The test strip components consist of: 1) a burgundy colored conjugate pad containing mouse anti-pHRP-II antibody conjugated with colloidal gold (pHRP II-gold conjugates), mouse anti-pLDH antibody conjugated with colloidal gold (pLDH-gold conjugates) and chicken IgY conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test bands (Pan and Pf bands) and a control band (C band). The Pan band is precoated with monoclonal anti-pLDH antibody by which the infection with any of the four species of plasmodia can be detected, the Pf band is pre-coated with polyclonal antipHRP- II antibodies for the detection of Pf infection, and the C band is coated with goat anti-Goat Anti Rabbit IgG. During the assay, an adequate volume of the blood specimen is dispensed into the sample well (S) of the test cassette, and a lysis buffer is added to the buffer well (B). The buffer contains a detergent that lyses the red blood cells and releases various plasmodium antigens which migrate by capillary action across the strip held in the cassette.

Kit components

Item	Specification
Detection Card	20T/40T
Sample Diluent	1 bottle
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Notes

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Please do not unpack the sealed pouch until you're ready to perform the test. If the package is obviously damaged, please do not use it.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15℃-30℃) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Hemolyzed blood may be used for the testing, but do not take precipitants.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as biohazardous waste.
11. The testing results should be read within 30 minutes after a specimen is applied to the sample well or sample well of the device. Read result after 30 minutes may give erroneous results.

12. Do not perform the test in a room with strong air flow, ie.an electric fan or strong air-conditioning.

Storage and expiry date

Storage: Store at 4-30℃. With cool and dry environment.

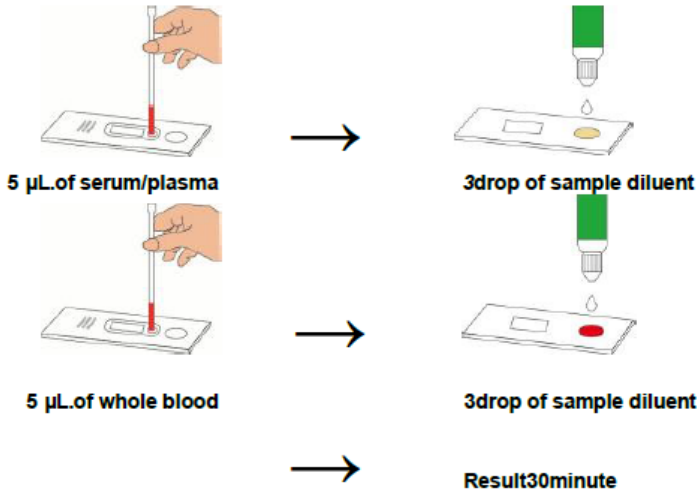
Expiry date: expiration date is on the packing box.

Requirements of sample

1. Consider any materials of human origin as infectious and handle them with standard bio-safety procedures.
2. Collect whole blood in a clean container containing anti-coagulant (EDTA, citrate or heparin) by venipuncture. Blood can be obtained by fingertip puncture as well.
3. Whole blood specimen should be stored in refrigeration (2 ~8 ℃) if not tested immediately for up to 3 days. The specimen should be frozen at -20 ℃ for longer storage. Avoid repeat freeze and thaw cycles.

Assay procedure

1. Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed. Blood will be hemolyzed after thawing.
2. When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.
3. Be sure to label the device with specimen's ID number.
4. Fill the blood transfer device (sample loop, mini plastic dropper or capillary tube) with the blood specimen not to exceed the specimen line as shown in the following images. The volume of the specimen is around 5 μL.
5. Practice a few times prior to testing if you are not familiar with the blood transfer device. For better precision, transfer specimen by pipette capable of delivering a 5μL volume.
   Holding the blood transfer device (sample loop, mini plastic dropper or capillary tube) vertically, dispense the entire specimen into the center of the sample well making sure that there are no air bubbles. Then add 3 drops (about 105-150 μL) of Lysis Buffer immediately.
6. Set up timer.
7. Results can be read in 30 minutes. It may take more than 20 minutes to have the background become clearer.
   Don't read result after 30 minutes. To avoid confusion, discard the test cassette after interpreting the result.



Note: This figure is for reference only.

Interpretation of results

1. **Negative result:** If only the C band is present, the absence of any burgundy color in both test bands (Pan and Pf) indicates that no plasmodium antigens are detected. The result is negative.

2. **Positive:**

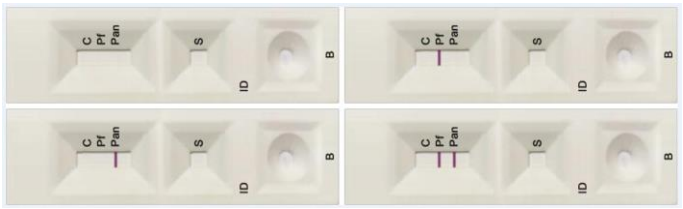
2.1 In addition to the presence of the C band, if only the Pan band is developed, the test indicates the presence of pLDH antigen. The result is either Pv, Pm, or Po positive.

2.2 In addition to the presence of the C band, if only the Pf band is developed, the test indicates the presence of pHRP-II antigen. The result is Pf positive.

2.3 Indicates the presence of both pHRP-II and pLDH. The result is both Pan and Pf positive (Subject Limitations of Test -3).

3. **Invalid:** If no C band is developed, the assay is invalid regardless of any burgundy color in the test

bands as indicated below. Repeat the assay with a new device.



Note: These figures are for reference only.

Limitations of this test method

1. The Test Procedure and Interpretation of Results must be followed closely when testing the presence of antigens to Malaria in serum, plasma and whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.
2. The Malaria Pf/Pan Antigen Test Kit is limited to the qualitative detection of plasmodium protozoa antigen in whole blood. The intensity of the test band does not have linear correlation with the antigen titer in the specimen.
3. In the case of co-infection with Pf and any of the other three plasmodia, both Pan and Pf bands will be developed. Thus, interpret the result cautiously when both Pan and Pf bands are visible.
4. A negative result for an individual subject indicates absence of detectable plasmodium protozoa antigen. However, a negative test result does not preclude the possibility of exposure to or infection with plasmodium protozoa.
5. A negative result can occur if the quantity of the plasmodium protozoa antigen present in the specimen is below the detection limits of the assay, or the antigens that are detected are not present during the stage of disease in which a sample is collected.
6. A recent study showed that due to their genetic diversity some Pf isolates collected in the Peruvian Amazon lack the HRP2 gene. Therefore, a negative Pf result but positive Pan result may not rule out infection of Pf in this area.
7. Some specimens containing an unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
8. The test result of this reagent can only be used as auxiliary tool. The test result should be combined with other data.